
Use of Genomics to Investigate Historical Importation of Shiga Toxin–Producing *Escherichia coli* Serogroup O26 and Nontoxigenic Variants into New Zealand

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Shiga toxin-producing *Escherichia coli* serogroup O26 is an important public health pathogen. Phylogenetic bacterial lineages in a country can be associated with the level and timing of international imports of live cattle, the main reservoir. We sequenced the genomes of 152 *E. coli* O26 isolates from New Zealand and compared them with 252 *E. coli* O26 genomes from 14 other countries. Gene variation among isolates from humans, animals, and food was strongly associated with country of origin and *stx* toxin profile but not isolation source. Time of origin estimates indicate serogroup O26 sequence type 21 was introduced at least 3 times into New Zealand from the 1920s to the 1980s, whereas nonvirulent O26 sequence type 29 strains were introduced during the early 2000s. New Zealand's remarkably fewer introductions of Shiga toxin-producing *Escherichia coli* O26 compared with other countries (such as Japan) might be related to patterns of trade in live cattle.

Shiga toxin-producing *Escherichia coli* (STEC) is an important public health pathogen, capable of causing hemorrhagic diarrhea and life-threatening kidney failure,

particularly in children (1). STEC is primarily transmitted through the fecal–oral route, and ruminants are important reservoirs of this zoonotic pathogen (2).

Initial research focused on STEC serotype O157:H7 as the main STEC pathogen involved in hemolytic uremic syndrome (3). However, STEC serogroup O26 has become an increasingly common cause of human disease. STEC O26 is the second most frequently detected serogroup causing STEC illness in New Zealand (4), the United States (5), and Europe (6).

Whole-genome sequencing (WGS) offers high-resolution identification of related bacterial isolates, while helping to direct source attribution investigations and interventions (7). The large amount of sequence data produced by such initiatives as GenomeTrakr (8) provides an opportunity to interpret the evolution and transmission of organisms across national boundaries.

New Zealand is a geographically isolated island nation that offers an opportunity to interpret the effects of importation and biosecurity measures on the control and transmission of zoonotic diseases (9). New Zealand has a relatively high incidence of notified human disease caused by STEC compared with other countries where the disease is notifiable; 11.9 STEC cases per 100,000 population were reported in New Zealand in 2017 (10), compared with 2.85 cases per 100,000 population in 2016 in the United States (11). A case–control study in New Zealand identified contact with animal manure and the presence of cattle in the local area as significant risk factors for human infection (12). After this case–control study, a New Zealand–wide cross-sectional study using

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DOI: <https://doi.org/10.3201/eid2503.180899>

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Table 1. Summary of 404 *Escherichia coli* serogroup O26 isolates in an investigation of the bacterium's historical importation into New Zealand

Country	stx Profile			Sequence type			Source				
	stx1	stx2	stx1 and stx2	No stx	ST21	ST29	Other	Human	Bovine	Food	Other animal
Australia, n = 1	1	0	0	0	1	0	0	1	0	0	0
Belgium, n = 24	20	1	2	1	20	1	3	16	8	0	0
Continental Europe,* n = 21	3	13	2	3	6	13	2	19	2	0	0
Japan, n = 94	70	8	11	5	88	5	1	77	16	0	1
New Zealand, n = 152	104	0	0	48	136	16	0	32	120	0	0
Other North America,† n = 4	3	0	0	1	3	0	1	2	1	1	0
United Kingdom, n = 29	10	7	8	4	25	3	1	28	1	0	0
United States, n = 79	60	9	5	5	66	10	3	45	27	4	3
Total, n = 404	271	38	28	67	345	48	11	220	174	5	5

*Denmark, n = 1; France, n = 9; Germany, n = 6; Italy, n = 1; Norway, n = 2; Poland, n = 1; Switzerland, n = 1.

†Canada, n = 3; Mexico, n = 1.

a culture-independent test found STEC O26 in 7.2% of young dairy calves sampled (13).

Our objective was to compare genomes of *E. coli* serogroup O26 isolates from human clinical cases and cattle in New Zealand with genomes of bacterial isolates from non-New Zealand sources, examining the genetic diversity and population structure, evolution, time to most recent common ancestor (tMRCA), antimicrobial resistance, and virulence genes. These data can be used to infer the probable importation, transmission, and evolution of STEC O26, which can inform risk management decisions with regard to movement of reservoir animals, as well as potential interventions for public health. This research received Massey University Ethics approval (Notification No. 4000016530).

Methods

New Zealand Bacterial Isolates: Selection, DNA and Library Preparation, and Sequencing

We conducted random stratified selection, by year, region, farm, and source, of 152 serogroup O26 bacterial isolates from New Zealand human sources (32 isolates) and bovine sources (120 isolates) from 1985 to 2016. We previously analyzed a subset of 66 bovine isolates as part of a cross-sectional study of STEC prevalence on dairy farms (13). We obtained human isolates from the Institute of Environmental Science and Research (Wallaceville, New Zealand) and bovine isolates from the Hopkirk Research Institute at Massey University (Palmerston North, New Zealand). We extracted DNA from a single colony picked

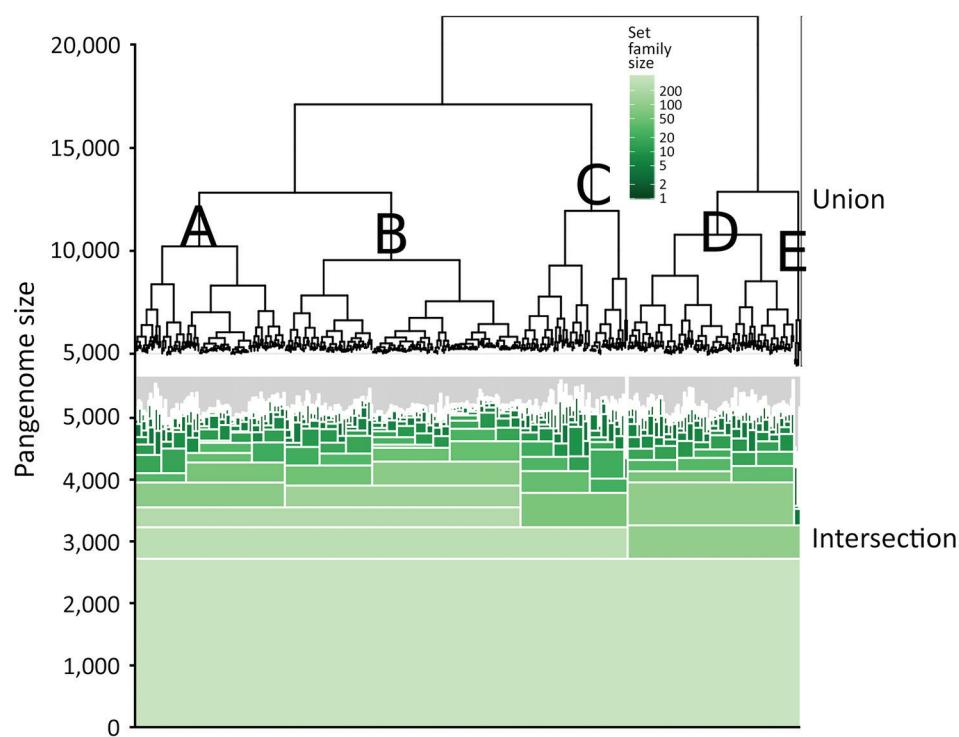


Figure 1. Hierarchical set analysis of 404 *Escherichia coli* serogroup O26 isolates in investigation of historical importation of Shiga toxin-producing *E. coli* serogroup O26 and nontoxicogenic variants into New Zealand, with a hierarchical set RaxML pangenome tree (top of figure) and shared gene groups visualized in green (bottom of figure). This figure illustrates shared gene groups after pangenome analysis. The union portion represents the pangenome relatedness between bacterial isolates. A–E indicate clades.

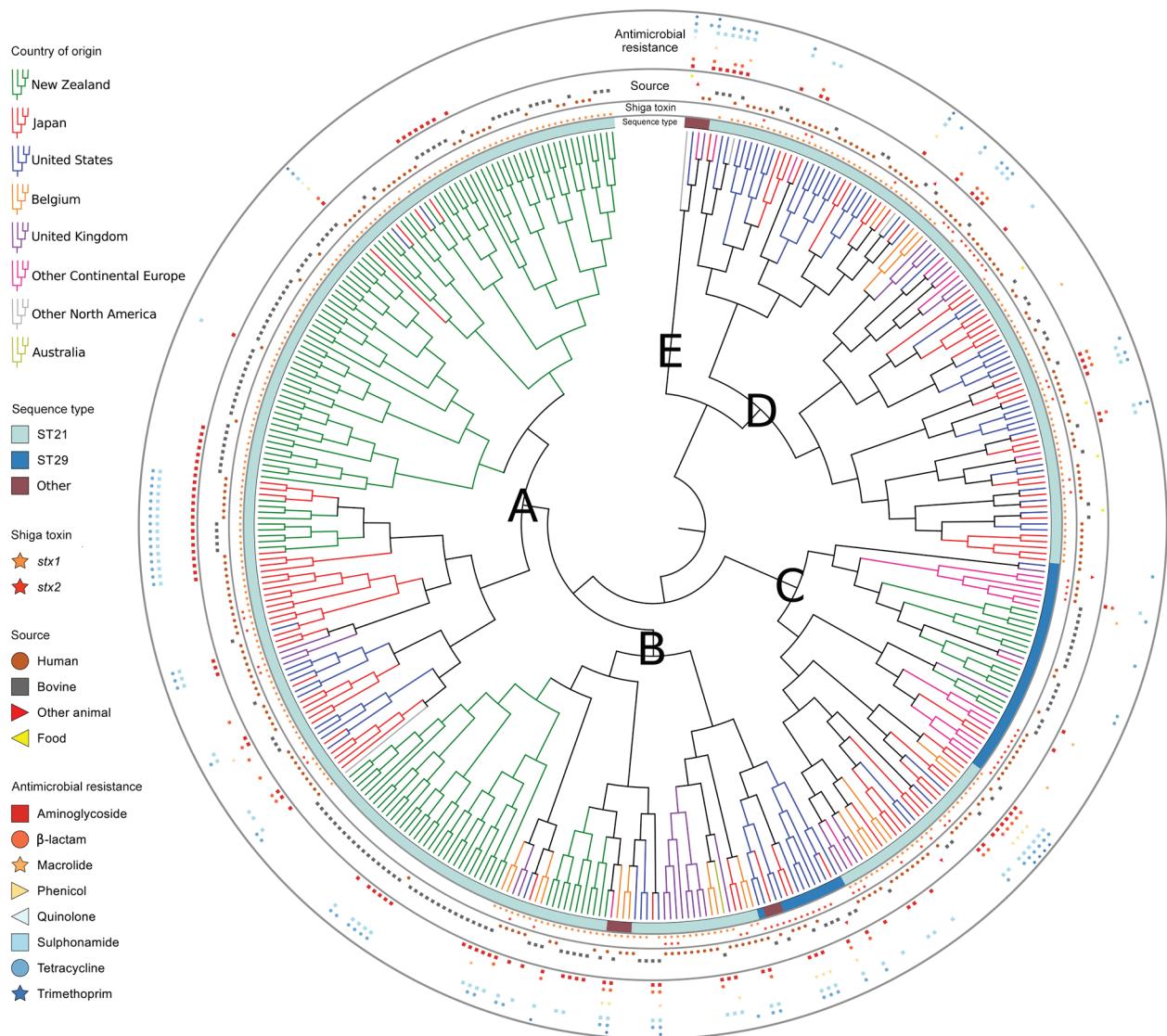


Figure 2. Hierarchical set RaxML tree of pangenome elements of 404 *Escherichia coli* serogroup O26 isolates in investigation of historical importation of Shiga toxin-producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand. A–E indicate clades, which are annotated. ST, sequence type.

from Columbia Horse Blood Agar (Fort Richard Laboratories, <http://www.fortrichard.com>) using the QIAamp DNA MiniKit (QIAGEN, <https://www.qiagen.com>) and prepared sequencing libraries using the Nextera XT DNA Library Preparation Kit (Illumina, <https://www.illumina.com>). Prepared libraries were submitted to New Zealand Genomics Limited (University of Otago, <https://www.otago.ac.nz/genomics/index.html>), which performed sequencing using Illumina MiSeq 2 × 250 bp PE or Illumina HiSeq 2 × 125 bp PE v4.

Processed reads are publicly available on the National Center for Biotechnology Information Sequence Read

Archive under BioProject ID PRJNA396667. Metadata are stored under BioSample accession nos. SAMN07430747–SAMN07430900 (Appendix 1 Table 1, <https://wwwnc.cdc.gov/EID/article/25/3/18-0899-App1.pdf>).

Selection and Retrieval of Publicly Available *E. coli* Serogroup O26 Raw Sequence Data

To standardize the downstream genomics comparison pipeline, we included only bacterial isolates with raw sequencing data in this study. Isolate selection was stratified by country, year, and isolation source; we randomly selected up to 4 isolates from the same country, year, and

Table 2. PERMANOVA analysis of *Escherichia coli* serogroup O26 pangenome genes and virulence genes in an investigation of the bacterium's historical importation into New Zealand*

Dataset, variable, no. genes	df	p value	Component of variation, %
Pangenome, n = 21,399			
Sequence type	5	0.0001	33
Country	14	0.0001	18
Isolation source	3	0.358	<0.01
stx profile	3	0.0001	6
Virulence genes, n = 192			
Sequence type	5	0.0001	83.7
Country	14	0.01	1.9
Isolation source	3	0.07	0.3
stx profile	3	0.0001	6.2

*This method determines whether the variation of the dataset is significantly associated with a particular variable. Residual variation: pangenome (43.0%), virulence genes (7.8%).

isolation source. Raw beef samples were classified as bovine, whereas the food classification indicated nonmeat samples (e.g., spinach, flour). All potential sequences for this study were selected in December 2017, and 2 corresponding authors of publicly available assembled genomes (14,15) provided unpublished raw sequence data (S. Dellanoy, French Agency for Food, Environmental and Occupational Health & Safety, pers. comm., 2017 Jan 12; C. Gabrielsen, St. Olavs University Hospital, pers. comm., 2017 Jan 18). All 252 publicly available serogroup O26 sequences selected for this study are listed in Appendix 1 Table 2.

Assembly, Annotation, and Initial Analyses of WGS Data

We used the Nullarbor pipeline in the accurate mode (16) to evaluate, assemble, and annotate the WGS data and EC-Typer to identify somatic (O) and flagellar (H) antigens (O:H serotype) (https://github.com/phac-nml/ecoli_serotyping). We identified virulence and resistance genes using ABRicate (<https://github.com/tseemann/abricate>), which bundles multiple databases for gene queries (Resfinder, CARD, ARG-ANNOT, NCBI BARRGD, NCBI, EcOH, PlasmidFinder, Ecoli_VF and VFDB). Identified attributes, metadata, virulence genes, and resistance genes for all genomes are provided in Appendix 2 (<https://wwwnc.cdc.gov/EID/article/25/3/18-0899-App2.xlsx>).

We performed pangenome analysis with the FindMyFriends package in the RStudio environment (17), which groups genes into orthologous clusters by implementing the cd-hit clustering algorithm (18), followed by a cluster refinement based on k-mer similarity. We examined pangenome composition using the HierarchicalSets package (19), estimating the similarity of isolates based on the number of shared (core) and characteristic (accessory/pan) genes. Gamma heterogeneity (the ratio of the number of core genes [intersect] to the number of pan genes [union]) was calculated for each group of genomes, and

isolates are hierarchically clustered to minimize total heterogeneity, producing a dendrogram representation of genomic similarity.

We generated RaxML maximum-likelihood trees using a general time-reversible model from the concatenated alignment of all core genes outputted by FindMyFriends (20). Then, we created a dissimilarity matrix with the virulence gene output, based on the presence or absence of virulence genes between pairs of isolates, and used it to create neighbor-joining trees.

We evaluated the pangenome similarity matrix, as well as a dissimilarity matrix of the 192 virulence genes, with PERMANOVA (PRIMER-E; Quest Research Limited, <https://www.primer-e.com>) by using sequence type (ST), country, isolation source, and stx profile as independent factors. Phylogenetic figures were created using the iTOL (Interactive Tree of Life) software (21), and further amended using Inkscape open source software version 0.92.2 (<https://inkscape.org>).

Single-Nucleotide Polymorphism Core Gene Alignment and tMRCA Analyses

We created a core gene alignment from the FindMyFriends package using DECIPHER (22). Two core alignments were performed for 2 STs: ST21 (345 isolates) and ST29 (48 isolates). Recombinant regions and identical isolates were removed using Gubbins 2.3.1 (23), resulting in 344 ST21 isolates and 48 ST29 isolates in the final analysis.

We determined the tMRCA using BEAST2 (24). The temporal signal was evaluated with BactDating (25) and found to be significant for both ST21 and ST29 data. Model evaluation of a combination of substitution, clock, and population models was performed using a method-of-moments estimator (26), and evaluation of log files using Tracer version 1.6.1 (<http://tree.bio.ed.ac.uk/software/tracer/>) led to a preferred model selection with the lowest AICM (Akaike's information criterion for Markov chain Monte Carlo) estimates and consistent tracer line. General time-reversible substitution models were used to estimate tMRCAAs with a coalescent extended Bayesian skyline model and relaxed molecular clock (27). tMRCA analysis was calibrated by tip dates (ST21, 1947–2017; ST29, 1952–2017); decimal dates were rounded to the middle of the month or year if an exact date was not available within the month or year. Effective sample size exceeded 100 for all models evaluated. Maximum-clade credibility trees were created using TreeAnnotator version 2.4.7 with a 10% burn-in (24). We determined the substitution rate for each ST, multiplying the substitution rate estimated by BEAST2 by the number of analyzed single-nucleotide polymorphisms (SNPs) and dividing the product by the mean genome size of the isolates analyzed.

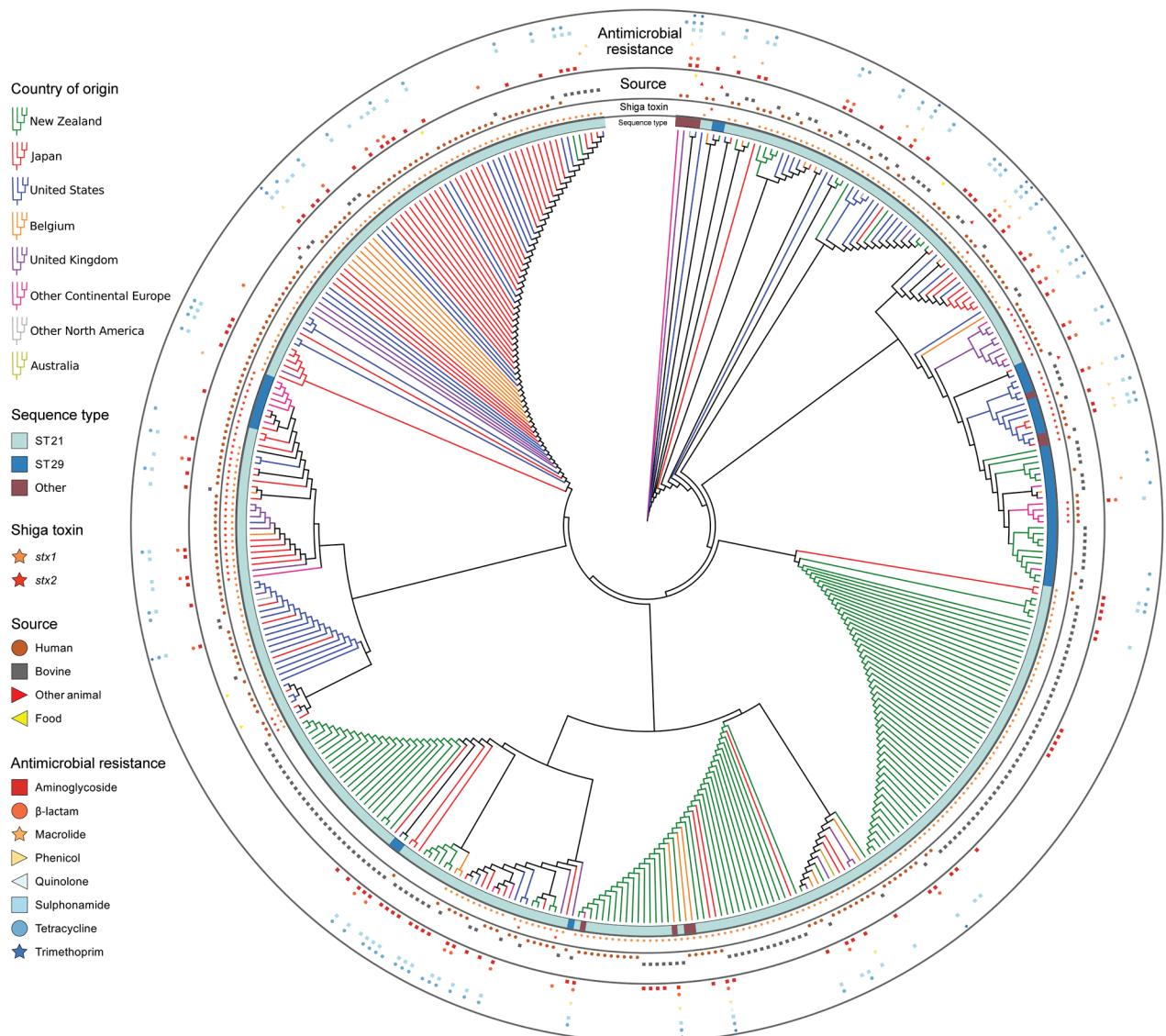


Figure 3. Neighbor-joining tree of 192 virulence genes of 404 *Escherichia coli* serogroup O26 isolates in investigation of historical importation of Shiga toxin-producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand. Branch lengths are ignored to better illustrate the country of origin of each isolate; therefore, closely spaced trellis-like branches have identical virulence profiles. ST, sequence type.

Cattle Importation Data

New Zealand cattle importation data were combined from a previous publication of historical importations of cattle into New Zealand (28) and Food and Agriculture Organization data from 1961 to 2013 (29). Live cattle imports into Japan from 1961 to 2013 were obtained from Food and Agriculture Organization data to enable us to compare them with New Zealand live cattle imports (29).

Results

Most genomes were obtained from New Zealand (152 genomes), Japan (94 genomes), and the United States (79 genomes) (Table 1). Most isolates were ST21 (345 isolates)

and ST29 (48 isolates); multiple *stx* gene profiles were represented (*stx*1, *stx*2, *stx*1, *stx*2, and no *stx*); and the source of isolates fell into 4 groups: human, bovine, food, and other animal.

Evolutionary Dynamics of *E. coli* Serogroup O26

Gene clustering analyses identified 2,718 core genes, 8,904 accessory genes, and 9,777 singleton genes, for a pangenome size of 21,399 genes. The pangenome was open and had a Heaps' Law coefficient of 0.35. Hierarchical clustering based on pangenome composition enabled the definition of 5 independent classes of isolates (A–E) for comparative purposes; group E contained

Table 3. Detection of antimicrobial resistance genes of 404 *Escherichia coli* serogroup O26 isolates in an investigation of the bacterium's historical importation into New Zealand*

Factor evaluated	Antimicrobial resistance, %							
	Aminoglycoside	β-lactam	Macrolide	Phenicol	Quinolone	Sulphonamide	Tetracycline	Trimethoprim
Country								
Australia, n = 1	100	0	0	100	0	100	0	0
Belgium, n = 24	67	25	4	17	0	67	42	17
Continental Europe, n = 21	29	14	10	5	0	24	14	0
Japan, n = 94	32	13	1	6	1	32	24	3
New Zealand, n = 152	26	1	1	0	0	12	12	1
Other North America, n = 4	50	25	25	0	25	50	100	0
United Kingdom, n = 29	24	21	0	4	0	29	25	4
United States, n = 79	13	13	0	4	0	4	14	3
Source*								
Human, n = 220	28	12	<1%	4	<1%	27	21	3
Bovine, n = 175	27	6	3	4	0	16	15	2
Food, n = 5	20	20	0	0	20	20	20	20
Total isolates, n = 404	30	10	2	4	1	23	19	3

*No antimicrobial resistance genes were detected in other animals (n = 5).

non-O26:H11 strains (Figure 1); clades A–D each share ≈4,000 core genes. A pangenome hierarchical set tree was annotated with country, ST, isolation source, and antimicrobial resistance gene class (Figure 2), with a real branch length figure available (Appendix 1 Figure 1). Multiple strains have circulated globally and are present in many countries.

PERMANOVA analysis for the pangenome and virulence genes revealed that gene variation among isolates was mostly explained by ST (pangenome, 33%; virulence genes, 84%), country of origin (pangenome, 18%; virulence genes, 2%), and *stx* profile (pangenome, 6%; virulence genes, 6%). Isolation source was not a significant factor (Table 2).

Pathogenicity and Antimicrobial Resistance of *E. coli* O26

A neighbor-joining tree based on a distance matrix of the presence and absence of virulence genes detected (n = 192) (Figure 3) shows that a large number of New Zealand isolates had identical virulence profiles from human and bovine sources; a large clade from Japan, the United States, and Belgium also has identical profiles. We compiled a real branch length figure (Appendix 1 Figure 2), the name and function of all 192 detected virulence genes (Appendix 1 Table 3), and the virulence genes detected for each genome (Appendix 2).

We detected resistance genes for 8 classes of antimicrobial drugs (Table 3; Appendix 2 Table). Resistance genes were detected in 252 (62.4%) bacterial isolates.

tMRCA Analysis and Inferred Global Importation and Transmission of *E. coli* O26

A core gene alignment of the 344 serogroup O26 ST21 isolates generated 9,702 SNPs, and the 48 ST29 isolates

generated 4,686 SNPs. In the tMRCA estimates for ST21 isolates (Figure 4) and ST29 isolates (Figure 5), important convergence dates were annotated with a 95% highest posterior density (HPD) interval (Appendix 1 Figures 3, 4). The calculated substitution rate for ST-21 was 1.4×10^{-7} substitutions/site/year (95% CI $1.1\text{--}1.7 \times 10^{-7}$ substitutions/site/year), and the substitution rate for ST29 isolates was 3.2×10^{-7} substitutions/site/year (95% CI $2.3\text{--}3.9 \times 10^{-7}$ substitutions/site/year).

Four New Zealand ST21 monophyletic clades indicate tMRCA estimates from the 1920s through the 1990s (Figure 4). Individual New Zealand monophyletic clades show evidence of importation from Europe (95% HPD interval 1958–1982) and more recently from the United States (95% HPD interval 1971–1992). Paraphyletic clades are visible from European sources, particularly from US and Japan isolates, which create a panmictic community, indicating frequent transmission between these countries. Two New Zealand ST29 monophyletic clades show tMRCA estimates from the late 1960s to the early 21st century (Figure 5). Japanese strains of 4 *stx2*-positive ST29 isolates appear to be closely related to strains from the United States and from Europe. Minimal evidence exists of transmission of New Zealand strains to the other countries evaluated in this study (Figures 4, 5).

Most live cattle imported into New Zealand arrived during the 1860s (Figure 6), and importations increased during the 1950s–1990s. New Zealand imported fewer cattle than Japan for all years examined; since 1991, New Zealand has consistently imported <100 live cattle per year, whereas Japan has imported >10,000 per year (Figure 7).

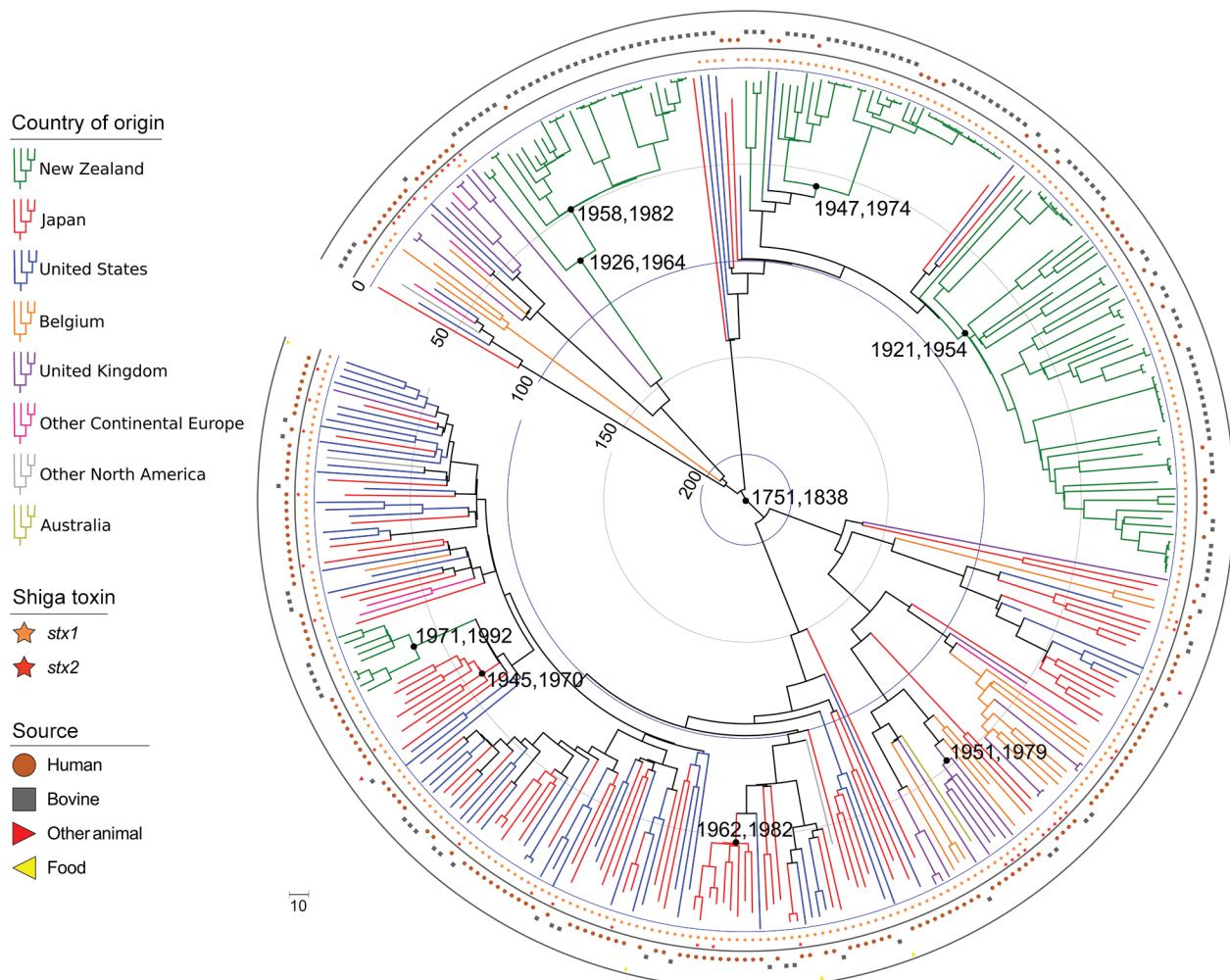


Figure 4. Maximum clade credibility tree of time of most recent common ancestor analysis of 344 *Escherichia coli* serogroup O26 sequence type 21 isolates in investigation of historical importation of Shiga toxin-producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand. Key convergence dates are annotated with 95% highest posterior density intervals, and the concentric circles indicate earlier time periods (blue, 100 years; gray, 50 years) from the age of the newest isolate (2017.5 in decimal years).

Discussion

We used WGS to compare New Zealand *E. coli* serogroup O26 bacteria with isolates from around the world. Our analyses demonstrated contrasting patterns of global panmixia and local isolation between different lineages of the O26 serogroup. Based on these patterns, we suggest that global O26 exchange most likely is linked to the import/export of live cattle; periods of between-country transmission occurred mainly during the 20th century. In New Zealand, several lineages have unique virulence profiles, each of which is likely to have been introduced at a single point in the recent past, and subsequently undergone local expansion and diversification. This country-specificity contrasts with other strains, notably from the United States and Japan, that appear to have been exchanged between geographic locations on multiple occasions over the same time.

Pangenome and virulence gene PERMANOVA analysis (Table 2) indicated that variation was best explained by multilocus subtype, country of origin, and *stx* profile. The lack of isolation source as a significant factor for pangenome or virulence gene analysis (Table 2) suggests that serogroup O26 isolates from humans, cattle, food, and other animals are not genetically differentiated and zoonotic transmission of this bacteria occurs frequently.

WGS analysis of STEC O157:H7 isolates in New Zealand showed findings similar to ours, where the within-country isolate diversity was unique and not related to the source of the isolate (30). As in our study, bovine and human New Zealand STEC O157:H7 isolates were closely related by genotyping, compared with isolates from the United States and Australia (30). Genes classified as virulent for humans are involved in the intestinal colonization of cattle

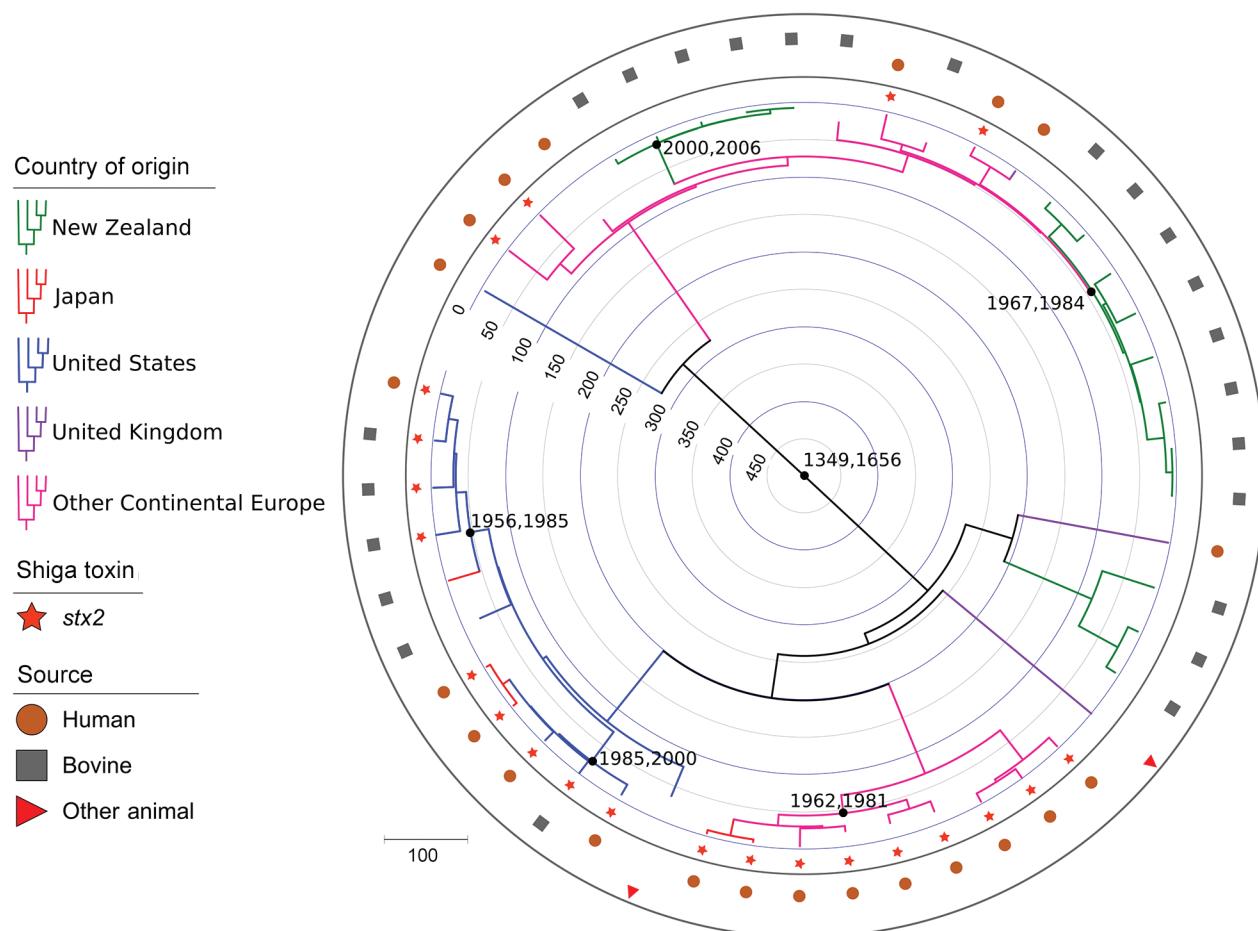


Figure 5. Maximum clade credibility tree of time of most recent common ancestor analysis of 48 *Escherichia coli* serogroup O26 sequence type 29 isolates in investigation of historical importation of Shiga toxin-producing *E. coli* serogroup O26 and nontoxigenic variants into New Zealand. Key convergence dates are annotated with 95% highest posterior density intervals, and concentric circles indicate prior time periods (blue, 100 years; gray, 50 years) from the age of the newest isolate (2017.0411 in decimal years).

(31,32); therefore, the contrasting virulence attributes associated with isolates from different countries might indicate separate niche adaptation and advantages for colonization of local host populations.

We observed that the *E. coli* O26 genome is open (Heaps' Law coefficient 0.35), meaning that the suite of genes possessed by each isolate is highly variable. These genes will be picked up from their local environments. The Public Goods Hypothesis proposes that the horizontal exchange of widely available DNA sequences is the primary driver for local bacterial evolution (33). This horizontal exchange of DNA will influence virulence profiles and other phenotypic traits, such as antimicrobial resistance. Applying this hypothesis to the evolution of *E. coli* O26 implies continued adaptation of the bacterial strains in local environments as they further acquire and share genes, which ultimately could lead to the emergence of new pathogenic lineages of STEC.

Evidence of relatively recent acquisition of *stx2* virulence within STEC O26 ST29 is a cause of concern (14). Non-STEC ST29 strains are present in cattle in New Zealand, but at the time this article was written, no STEC O26 with the *stx2* virulence gene had been reported there. The emergence of highly pathogenic strains that harbor the *stx2* toxin gene has led to an increase in hemolytic uremic syndrome related to the O26 serogroup (14,34). Serogroup O26 ST29 *stx2* isolates also have been identified in Japan (35), and 2 separate clades of *stx2* ST29 isolates from Japan may have been imported from the United States and Europe (Figure 5). The lack of highly pathogenic ST29 *stx2* isolates in New Zealand might be due to few live cattle importations, as well as no major horizontal genetic transfer events of *stx2* to *E. coli* O26 in New Zealand.

The resistance profiles form distinct combinations of resistance genes in isolates from particular countries (Figures 2, 3). Antimicrobial drugs are not usually prescribed

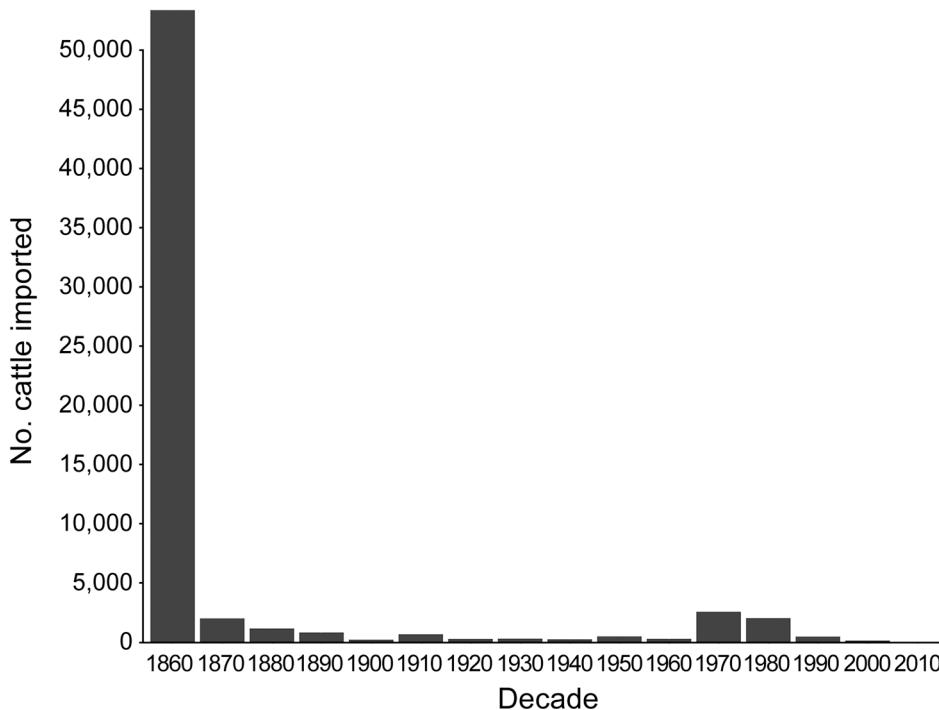


Figure 6. Historical importations of live cattle into New Zealand, 1860–2010.

for human STEC infections (36); however, selection pressure from antimicrobial drug use in livestock and humans with undiagnosed diarrheal illness may influence the evolution of resistance. Antimicrobial resistance from all human isolates was higher or equal to that of bovine isolates (Table 3), with the exception of that to macrolides.

The tMRCA of New Zealand *E. coli* O26 clades suggests several separate importations of strains that appear to coincide with cattle importation events (Figures 4–6). Phylogenetic analyses suggest certain New Zealand clades are more associated with specific geographic areas

(e.g., United States or continental Europe), indicating that transmission pathways are likely to exist through live animal imports. The estimated substitution rates for ST21 and ST29 isolates in the present study are similar to previous estimates for serogroup O26 ($2.8\text{--}4.3 \times 10^{-7}$ substitutions/site/year) (37) and O157:H7 (38). The tMRCA of all ST21 (1751–1838) (Figure 4) is similar to tMRCA estimates of ≈ 213 years ago for a large ST21 clade evaluated by Ogura et al. (37).

We observed a remarkable difference between 2 island nations with the most bacterial isolates analyzed: New

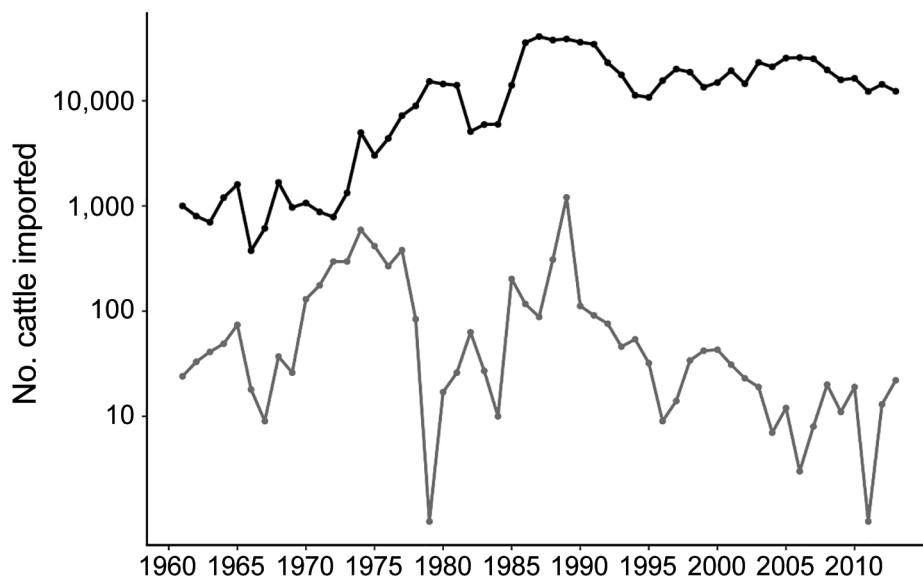


Figure 7. Comparison of live cattle imported (\log_{10} scale) into New Zealand and Japan during 1961–2013. Japan, black; New Zealand, gray.

Zealand ($n = 152$) and Japan ($n = 94$). In the Hierarchical-Sets pangenome (Figure 2), virulence gene (Figure 3), and BEAST2 ST21 (Figure 4) analyses, New Zealand isolates show monophyletic clades, whereas Japan isolates are paraphyletic with US isolates. The historically larger number of live cattle importations into Japan than into New Zealand (Figure 7) might explain this difference. Japan was a major importer of US live cattle during the second half of the 20th century, until the detection of bovine spongiform encephalopathy led to a ban on all live cattle from the United States in 2003; current importations come from Australia (39,40). The relatively large number of imported live cattle in Japan could explain the different population structure of *E. coli* O26 in New Zealand and Japan.

Our dataset enables minimal interpretation of open border areas, such as the European Union or countries in the North American Free Trade Agreement (Mexico, Canada, and the United States), but our results from New Zealand suggest the introduction of serogroup O26 bacterial strains occurred during periods of intensive cattle importation. In cattle, STEC is a commensal bacterium and is shed intermittently (41); therefore, testing cattle before transportation is unrealistic. Our tMRCA and phylogenetic analyses suggest that minimal exchange of strains has occurred between countries in the 21st century; however, continued movement of cattle across international borders is likely to continue to influence the spread and genetic diversity of STEC around the world.

The results of our study are subject to several limitations. The quantity and diversity of *E. coli* O26 isolates from other countries were variable. More *E. coli* O26 isolates from Australia would have enabled us to better compare the effect of importation of cattle into New Zealand because Australia was the source of many historical cattle importations (28). Sequence data were more common from the past few years, and mostly human isolates were available. Although we randomly selected our New Zealand isolates from human and bovine isolates spanning >30 years from a diverse geographic range in New Zealand, some isolates were from the same farm (Appendix 1 Table 1), leading to a potential bias. Although non-STEC and STEC strains of the same serogroup are commonly of different lineages (42), our focus on a defined O surface antigen (O26) to classify bacterial isolates and evaluate evolutionary and phylogenetic relationships is consistent with other studies (30,37).

Our results suggest worldwide dissemination of multiple strains of ST21 and ST29 STEC and nontoxigenic serogroup O26 lineages occurred during the 20th century. Close genetic similarities between *E. coli* O26 isolated from multiple different sources indicates common transmission pathways among animals, food sources, and humans. The limited introductions of *E. coli* O26 strains into

New Zealand are most likely linked to minimal importations of live cattle.

Further sequencing of historical isolates from multiple sources will improve evolutionary and epidemiologic studies. Full use of the genomic information of STEC will require a coordinated international approach to sequencing, data curation, analysis, and interpretation of those data (43). Although it is difficult to directly attribute transmission and emergence of STEC strains based on global historical events, interpreting evolutionary genomic data against economic and sociopolitical factors can help determine the drivers of pathogen emergence and dissemination, and inform future public health policy.

Acknowledgments

We thank Sabine Delannoy and Christina Gabrielsen for providing raw sequence data from their published research and the NeSI high-performance computing facilities provided by the New Zealand eScience Infrastructure. We extend a special thank you to the scientists and computing professionals all over the world who publicly provide and maintain WGS data that can be used for public health research.

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Use of Genomics to Investigate Historical Importation of Shiga Toxin–Producing *Escherichia coli* Serogroup O26 and Nontoxigenic Variants into New Zealand

Appendix 1

Appendix 1 Table 1. Metadata for New Zealand isolates (BioProject PRJNA396667), including FarmID for isolates retrieved from the same farm

Sample_name	Accession	Instrument	Collection_date	Geo_loc_name	Isolation_source	Host_age	Serotype	Coverage	FarmID
100ST2	SAMN07430826	Illumina HiSeq 2500	1-Sep-08	New Zealand: Taranaki	Bovine	Calf	O26:H11	87x	
11ST	SAMN07430827	Illumina HiSeq 2501	19-Aug-08	New Zealand: Taranaki	Bovine	Calf	O26:H11	77x	
129ST2	SAMN07430779	Illumina HiSeq 2502	5-Sep-08	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	100x	
14ER2056	SAMN07430858	Illumina HiSeq 2503	12-Jul-14	New Zealand: Waikato	Human		O26:H11	112x	
14ER2240	SAMN07430859	Illumina HiSeq 2504	31-Jul-14	New Zealand: Waikato	Human		O26:H11	98x	
14ER3837	SAMN07430789	Illumina HiSeq 2505	13-Nov-14	New Zealand: Nelson Marlborough	Human		O26:H11	71x	
15ER0146	SAMN07430790	Illumina HiSeq 2506	7-Jan-15	New Zealand: Nelson Marlborough	Human		O26:H11	95x	
15ER0512	SAMN07430775	Illumina HiSeq 2507	3-Feb-15	New Zealand: Combined Auckland	Human		O26:H11	83x	
15ER0857	SAMN07430747	Illumina HiSeq 2508	27-Feb-15	New Zealand: Auckland	Human		O26:H11	80x	
15ER2552	SAMN07430776	Illumina HiSeq 2509	12-Aug-15	New Zealand: Combined Auckland	Human		O26:H11	118x	
15ER2837	SAMN07430748	Illumina HiSeq 2510	28-Aug-15	New Zealand: Auckland	Human		O26:H11	84x	
15ER3334	SAMN07430754	Illumina HiSeq 2511	26-Sep-15	New Zealand: Canterbury	Human		O26:H11	84x	
15ER3567	SAMN07430791	Illumina HiSeq 2512	13-Oct-15	New Zealand: Nelson Marlborough	Human		O26:H11	104x	
15ER3804	SAMN07430900	Illumina HiSeq 2513	1-Nov-15	New Zealand: Auckland	Human		O26:H11	94x	
15ER4241	SAMN07430749	Illumina HiSeq 2514	7-Dec-15	New Zealand: Auckland	Human		O26:H11	93x	
16ER0243	SAMN07430795	Illumina HiSeq 2515	14-Jan-16	New Zealand: Northland	Human		O26:H11	90x	
16ER0248	SAMN07430792	Illumina HiSeq 2516	13-Jan-16	New Zealand: Nelson Marlborough	Human		O26:H11	77x	
16ER0520	SAMN07430777	Illumina HiSeq 2517	4-Feb-16	New Zealand: Combined Auckland	Human		O26:H11	94x	
16ER0672	SAMN07430750	Illumina HiSeq 2518	2016	New Zealand: Auckland	Human		O26:H11	99x	
16ER0946	SAMN07430800	Illumina HiSeq 2519	4-Mar-16	New Zealand: Southland	Human		O26:H11	100x	
16ER1209	SAMN07430796	Illumina HiSeq 2520	23-Mar-16	New Zealand: Northland	Human		O26:H11	103x	

Sample_name	Accession	Instrument	Collection_date	Geo_loc_name	Isolation_source	Host_age	Serotype	Coverage	FarmID
16ER1432	SAMN07430751	Illumina HiSeq 2521	9-Apr-16	New Zealand: Auckland	Human		O26:H11	105x	
16ER1646	SAMN07430778	Illumina HiSeq 2522	30-Apr-16	New Zealand: Combined Auckland	Human		O26:H11	79x	
16ER1892	SAMN07430860	Illumina HiSeq 2523	20-May-16	New Zealand: Waikato	Human		O26:H11	91x	
16ER1973	SAMN07430793	Illumina HiSeq 2524	26-May-16	New Zealand: Nelson Marlborough	Human		O26:H11	109x	
16ER2139	SAMN07430861	Illumina HiSeq 2525	18-Jun-16	New Zealand: Waikato	Human		O26:H11	75x	
191ST2	SAMN07430828	Illumina HiSeq 2526	18-Sep-08	New Zealand: Taranaki	Bovine	Calf	O26:H11	104x	
22ST2	SAMN07430780	Illumina HiSeq 2527	19-Aug-08	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	81x	
66ST1	SAMN07430829	Illumina HiSeq 2528	27-Aug-08	New Zealand: Taranaki	Bovine	Calf	O26:H11	104x	
A14a	SAMN07430755	Illumina MiSeq	17-Aug-09	New Zealand: Canterbury	Bovine	Calf	O26:H11	164x	
A17a	SAMN07430756	Illumina MiSeq	17-Aug-09	New Zealand: Canterbury	Bovine	Calf	O26:H11	117x	
a185c	SAMN07430757	Illumina HiSeq 2531	19-Jan-11	New Zealand: Canterbury	Bovine	Cow	O26:H11	230x	
a234d	SAMN07430758	Illumina HiSeq 2532	15-Jun-11	New Zealand: Canterbury	Bovine	Cow	O26:H11	120x	
A46a	SAMN07430759	Illumina HiSeq 2533	30-Jul-10	New Zealand: Canterbury	Bovine	Calf	O26:H11	94x	
A65a	SAMN07430760	Illumina HiSeq 2534	16-Aug-10	New Zealand: Canterbury	Bovine	Calf	O26:H11	81x	
A65b	SAMN07430761	Illumina HiSeq 2535	16-Aug-10	New Zealand: Canterbury	Bovine	Calf	O26:H11	104x	
A87b	SAMN07430762	Illumina HiSeq 2536	6-Sep-10	New Zealand: Canterbury	Bovine	Calf	O26:H11	104x	
AGR373	SAMN07430781	Illumina HiSeq 2537	12-Dec-02	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	110x	
AGR72	SAMN07430782	Illumina HiSeq 2538	21-Nov-02	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	111x	
e171b	SAMN07430831	Illumina HiSeq 2539	22-Feb-11	New Zealand: Taranaki	Bovine	Cow	O26:H11	110x	
e171c	SAMN07430832	Illumina HiSeq 2540	22-Feb-11	New Zealand: Taranaki	Bovine	Cow	O26:H11	100x	
e186a	SAMN07430833	Illumina HiSeq 2541	22-Mar-11	New Zealand: Taranaki	Bovine	Cow	O26:H11	102x	
E189b	SAMN07430834	Illumina HiSeq 2542	13-Sep-10	New Zealand: Taranaki	Bovine	Calf	O26:H11	73x	
e195b	SAMN07430835	Illumina HiSeq 2543	18-Apr-11	New Zealand: Taranaki	Bovine	Cow	O26:H11	114x	
e26a	SAMN07430836	Illumina HiSeq 2544	22-Dec-09	New Zealand: Taranaki	Bovine	Cow	O26:H11	68x	
e37c	SAMN07430837	Illumina HiSeq 2545	18-Jan-10	New Zealand: Taranaki	Bovine	Cow	O26:H11	89x	
E46a	SAMN07430838	Illumina HiSeq 2546	25-Aug-09	New Zealand: Taranaki	Bovine	Calf	O26:H11	105x	
E59b	SAMN07430839	Illumina HiSeq 2547	7-Sep-09	New Zealand: Taranaki	Bovine	Calf	O26:H11	97x	
ER005420	SAMN07430854	Illumina HiSeq 2548	2000	New Zealand: Unknown	Human		O26:H11	106x	
ER854674	SAMN07430855	Illumina HiSeq 2549	1985	New Zealand: Unknown	Human		O26:H11	100x	
ER985544	SAMN07430856	Illumina HiSeq 2550	1998	New Zealand: Unknown	Human		O26:H11	83x	
ERL023841	SAMN07430857	Illumina HiSeq 2551	2002	New Zealand: Unknown	Human		O26:H11	96x	
ERL071565	SAMN07430794	Illumina HiSeq 2552	10-May-07	New Zealand: Nelson Marlborough	Human		O26:H11	109x	
ERL093655	SAMN07430752	Illumina HiSeq 2553	31-Oct-09	New Zealand: Auckland	Human		O26:H11	87x	
ERL111686	SAMN07430763	Illumina HiSeq 2554	6-May-11	New Zealand: Canterbury	Human		O26:H11	78x	

Sample_name	Accession	Instrument	Collection_date	Geo_loc_name	Isolation_source	Host_age	Serotype	Coverage	FarmID
ERL121992	SAMN07430797	Illumina HiSeq 2555	20-Feb-12	New Zealand: Northland	Human		O26:H11	95x	
H108a	SAMN07430798	Illumina MiSeq	21-Aug-09	New Zealand: Northland	Bovine	Calf	O26:H11	145x	
H113a	SAMN07430799	Illumina MiSeq	21-Aug-09	New Zealand: Northland	Bovine	Calf	O26:H11	143x	
H132a	SAMN07430862	Illumina HiSeq 2558	21-Aug-09	New Zealand: Waikato	Bovine	Calf	O26:H11	75x	
H13ESR01843	SAMN07430753	Illumina HiSeq 2559	20-Mar-13	New Zealand: Auckland	Human		O26:H11	103x	
h148a	SAMN07430863	Illumina HiSeq 2560	21-Feb-11	New Zealand: Waikato	Bovine	Cow	O26:H11	86x	
h148b	SAMN07430864	Illumina HiSeq 2561	21-Feb-11	New Zealand: Waikato	Bovine	Cow	O26:H11	71x	
h199a	SAMN07430865	Illumina HiSeq 2562	14-Jun-11	New Zealand: Waikato	Bovine	Cow	O26:H11	75x	
h199c	SAMN07430866	Illumina HiSeq 2563	14-Jun-11	New Zealand: Waikato	Bovine	Cow	O26:H11	67x	
H31c	SAMN07430867	Illumina HiSeq 2564	28-Jul-09	New Zealand: Waikato	Bovine	Calf	O26:H11	84x	
Hide14d	SAMN07430868	Illumina HiSeq 2565	10-Aug-10	New Zealand: Waikato	Bovine	Calf	O26:H11	120x	
ND29	SAMN07430869	Illumina HiSeq 2566	6-Nov-09	New Zealand: Waikato	Bovine	Calf	O26:H11	129x	
ND3	SAMN07430870	Illumina HiSeq 2567	21-Oct-09	New Zealand: Waikato	Bovine	Calf	O26:H11	125x	
ND35	SAMN07430871	Illumina HiSeq 2568	12-Nov-09	New Zealand: Waikato	Bovine	Calf	O26:H11	107x	
ND62	SAMN07430872	Illumina HiSeq 2569	24-Nov-09	New Zealand: Waikato	Bovine	Calf	O26:H11	119x	
Pre15a	SAMN07430873	Illumina HiSeq 2570	26-Jul-10	New Zealand: Waikato	Bovine	Calf	O26:H11	113x	
Pre4d	SAMN07430874	Illumina HiSeq 2571	3-Aug-10	New Zealand: Waikato	Bovine	Calf	O26:H11	115x	
T11i	SAMN07430801	Illumina HiSeq 2572	1-Sep-09	New Zealand: Southland	Bovine	Calf	O26:H11	95x	
t126c	SAMN07430802	Illumina HiSeq 2573	21-Sep-10	New Zealand: Southland	Bovine	Cow	O26:H11	93x	
t128a	SAMN07430803	Illumina HiSeq 2574	21-Dec-10	New Zealand: Southland	Bovine	Cow	O26:H11	98x	
t173a	SAMN07430804	Illumina HiSeq 2575	20-Apr-11	New Zealand: Southland	Bovine	Cow	O26:H11	165x	
t25a	SAMN07430805	Illumina HiSeq 2576	17-Dec-09	New Zealand: Southland	Bovine	Cow	O26:H11	101x	
t25c	SAMN07430806	Illumina HiSeq 2577	17-Dec-09	New Zealand: Southland	Bovine	Cow	O26:H11	105x	
T27a	SAMN07430807	Illumina MiSeq	14-Sep-09	New Zealand: Southland	Bovine	Calf	O26:H11	142x	
T48b	SAMN07430808	Illumina HiSeq 2579	17-Aug-10	New Zealand: Southland	Bovine	Calf	O26:H11	109x	
T66c	SAMN07430809	Illumina HiSeq 2580	17-Aug-10	New Zealand: Southland	Bovine	Calf	O26:H11	125x	
VC1113e	SAMN07430840	Illumina HiSeq 2581	4-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	73x	VCF75
VC1122f	SAMN07430841	Illumina MiSeq	4-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	129x	VCF76
VC1125e	SAMN07430842	Illumina HiSeq 2583	4-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	95x	VCF76
VC1139e	SAMN07430764	Illumina MiSeq	11-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	114x	VCF77
VC1140e	SAMN07430765	Illumina HiSeq 2585	11-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	90x	VCF77
VC1186e	SAMN07430766	Illumina HiSeq 2586	15-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	81x	VCF80
VC1187e	SAMN07430767	Illumina HiSeq 2587	15-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	125x	VCF80
VC1190e	SAMN07430768	Illumina HiSeq 2588	15-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	81x	VCF81

Sample_name	Accession	Instrument	Collection_date	Geo_loc_name	Isolation_source	Host_age	Serotype	Coverage	FarmID
VC1195e	SAMN07430769	Illumina HiSeq 2589	15-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	78x	VCF81
VC1196e	SAMN07430770	Illumina HiSeq 2590	15-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	93x	VCF81
VC1202e	SAMN07430771	Illumina HiSeq 2591	15-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	64x	VCF81
VC1309e	SAMN07430772	Illumina HiSeq 2592	17-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	79x	VCF89
VC1310e	SAMN07430773	Illumina HiSeq 2593	17-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	158x	VCF89
VC1311e	SAMN07430774	Illumina HiSeq 2594	17-Sep-14	New Zealand: Canterbury	Bovine	Calf	O26:H11	75x	VCF89
VC1362e	SAMN07430810	Illumina HiSeq 2595	22-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	112x	VCF93
VC1366e	SAMN07430811	Illumina HiSeq 2596	22-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	57x	VCF93
VC1367e	SAMN07430812	Illumina HiSeq 2597	22-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	87x	VCF93
VC1394e	SAMN07430813	Illumina MiSeq	23-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	97x	VCF95
VC1395e	SAMN07430814	Illumina HiSeq 2599	23-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	45x	VCF95
VC1395f	SAMN07430815	Illumina HiSeq 2600	23-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	102x	VCF95
VC1395g	SAMN07430816	Illumina HiSeq 2601	23-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	71x	VCF95
VC1396e	SAMN07430817	Illumina HiSeq 2602	23-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	111x	VCF95
VC1403e	SAMN07430818	Illumina HiSeq 2603	23-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	143x	VCF95
VC1471e	SAMN07430819	Illumina MiSeq	24-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	124x	VCF100
VC1471f	SAMN07430820	Illumina HiSeq 2605	24-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	136x	VCF100
VC1471g	SAMN07430821	Illumina HiSeq 2606	24-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	70x	VCF100
VC1471h	SAMN07430822	Illumina HiSeq 2607	24-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	70x	VCF100
VC1473e	SAMN07430823	Illumina HiSeq 2608	24-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	126x	VCF100
VC1474e	SAMN07430824	Illumina HiSeq 2609	24-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	97x	VCF100
VC1486e	SAMN07430825	Illumina HiSeq 2610	24-Sep-14	New Zealand: Southland	Bovine	Calf	O26:H11	75x	VCF101
VC396e	SAMN07430875	Illumina HiSeq 2611	12-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	178x	VCF27
VC397e	SAMN07430876	Illumina HiSeq 2612	12-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	51x	VCF27
VC401e	SAMN07430877	Illumina HiSeq 2613	12-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	65x	VCF31
VC452e	SAMN07430878	Illumina HiSeq 2614	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	70x	VCF31
VC456e	SAMN07430879	Illumina HiSeq 2615	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	84x	VCF31

Sample_name	Accession	Instrument	Collection_date	Geo_loc_name	Isolation_source	Host_age	Serotype	Coverage	FarmID
VC459e	SAMN07430880	Illumina HiSeq 2616	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	75x	VCF31
VC473e	SAMN07430881	Illumina HiSeq 2617	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	71x	VCF32
VC474e	SAMN07430882	Illumina HiSeq 2618	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	88x	VCF32
VC474f	SAMN07430883	Illumina HiSeq 2619	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	69x	VCF32
VC474g	SAMN07430884	Illumina HiSeq 2620	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	65x	VCF32
VC474h	SAMN07430885	Illumina HiSeq 2621	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	75x	VCF32
VC476e	SAMN07430886	Illumina HiSeq 2622	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	88x	VCF32
VC479e	SAMN07430888	Illumina HiSeq 2623	13-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	61x	VCF32
VC545e	SAMN07430889	Illumina HiSeq 2624	18-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	91x	VCF35
VC547e	SAMN07430890	Illumina HiSeq 2625	18-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	68x	VCF35
VC550e	SAMN07430891	Illumina HiSeq 2626	18-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	105x	VCF35
VC554e	SAMN07430892	Illumina HiSeq 2627	18-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	104x	VCF35
VC555e	SAMN07430893	Illumina HiSeq 2628	18-Aug-14	New Zealand: Waikato	Bovine	Calf	O26:H11	77x	VCF35
VC833e	SAMN07430783	Illumina MiSeq	27-Aug-14	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	117x	VCF56
VC833f	SAMN07430784	Illumina HiSeq 2630	27-Aug-14	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	62x	VCF56
VC833g	SAMN07430785	Illumina HiSeq 2631	27-Aug-14	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	98x	VCF56
VC833h	SAMN07430786	Illumina HiSeq 2632	27-Aug-14	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	52x	VCF56
VC836e	SAMN07430787	Illumina HiSeq 2633	27-Aug-14	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	61x	VCF56
VC837e	SAMN07430788	Illumina HiSeq 2634	27-Aug-14	New Zealand: Manawatu-Wellington	Bovine	Calf	O26:H11	78x	VCF56
VC880e	SAMN07430843	Illumina HiSeq 2635	1-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	54x	VCF59
VC932f	SAMN07430844	Illumina HiSeq 2636	1-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	74x	VCF63
VC936e	SAMN07430845	Illumina HiSeq 2637	1-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	97x	VCF63
VC940e	SAMN07430846	Illumina HiSeq 2638	1-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	102x	VCF63
VC943e	SAMN07430847	Illumina MiSeq	2-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	115x	VCF64
VC943f	SAMN07430848	Illumina HiSeq 2640	2-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	84x	VCF64
VC943g	SAMN07430849	Illumina HiSeq 2641	2-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	102x	VCF64
VC943h	SAMN07430850	Illumina HiSeq 2642	2-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	64x	VCF64
VC946e	SAMN07430851	Illumina HiSeq 2643	2-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	101x	VCF64
VC951e	SAMN07430852	Illumina HiSeq 2644	2-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	54x	VCF64
VC955e	SAMN07430853	Illumina HiSeq 2645	2-Sep-14	New Zealand: Taranaki	Bovine	Calf	O26:H11	74x	VCF64
VL0828h	SAMN07430894	Illumina HiSeq 2646	20-Aug-15	New Zealand: Waikato	Bovine	Calf	O26:H11	94x	
VL0958f	SAMN07430895	Illumina HiSeq 2647	1-Sep-15	New Zealand: Waikato	Bovine	Calf	O26:H11	98x	
VL1058g	SAMN07430896	Illumina HiSeq 2648	7-Sep-15	New Zealand: Waikato	Bovine	Calf	O26:H11	145x	
VL1277e	SAMN07430897	Illumina HiSeq 2649	12-Jul-16	New Zealand: Waikato	Bovine	Calf	O26:H11	124x	
VL2020e	SAMN07430898	Illumina HiSeq 2650	9-Aug-16	New Zealand: Waikato	Bovine	Calf	O26:H11	112x	
VL2228e	SAMN07430899	Illumina HiSeq 2651	29-Aug-16	New Zealand: Waikato	Bovine	Calf	O26:H11	135x	

Appendix 1 Table 2. Bioproject, BioSample, SRA run number, country, source, and unique identifier for all public sequence data used in this study (n = 252)

BioProject	BioSample	SRA run	Country	Source	Current_study_ID
PRJNA230969	SAMN05607363	SRR5330941	Australia	Human	MOD1EC1684_Australia_human
PRJDB5571	SAMD00075768	DRR103425	Belgium	Bovine	357S89_Belgium_bovine
PRJDB5571	SAMD00075735	DRR103392	Belgium	Human	EH031_Belgium_human
PRJDB5571	SAMD00075761	DRR103418	Belgium	Bovine	631KH91_Belgium_bovine
PRJDB5571	SAMD00075766	DRR103423	Belgium	Human	EH182_Belgium_human
PRJDB5571	SAMD00075767	DRR103424	Belgium	Human	EH193_Belgium_human
PRJDB5571	SAMD00075736	DRR103393	Belgium	Human	EH322_Belgium_human
PRJDB5571	SAMD00075764	DRR103421	Belgium	Bovine	B44_Belgium_bovine
PRJDB5571	SAMD00075763	DRR103420	Belgium	Bovine	B43_Belgium_bovine
PRJDB5571	SAMD00075771	DRR103428	Belgium	Bovine	11KH263_Belgium_bovine
PRJDB5571	SAMD00075769	DRR103426	Belgium	Bovine	11KH63_Belgium_bovine
PRJDB5571	SAMD00075770	DRR103427	Belgium	Bovine	11KH245_Belgium_bovine
PRJDB5571	SAMD00075971	DRR103628	Belgium	Human	EH2035_Belgium_human
PRJDB5571	SAMD00075976	DRR103633	Belgium	Human	EH2083_Belgium_human
PRJDB5571	SAMD00075975	DRR103632	Belgium	Human	EH2075_Belgium_human
PRJDB5571	SAMD00075974	DRR103631	Belgium	Human	EH2068_Belgium_human
PRJDB5571	SAMD00075772	DRR103429	Belgium	Bovine	12KH23_Belgium_bovine
PRJDB5571	SAMD00075981	DRR103638	Belgium	Human	EH2208_Belgium_human
PRJDB5571	SAMD00075984	DRR103641	Belgium	Human	EH2244_Belgium_human
PRJDB5571	SAMD00075983	DRR103640	Belgium	Human	EH2219_Belgium_human
PRJDB5571	SAMD00075982	DRR103639	Belgium	Human	EH2209_Belgium_human
PRJDB5571	SAMD00075988	DRR103645	Belgium	Human	EH2258_Belgium_human
PRJDB5571	SAMD00075986	DRR103643	Belgium	Human	EH2252_Belgium_human
PRJDB5571	SAMD00075987	DRR103644	Belgium	Human	EH2257_Belgium_human
PRJDB5571	SAMD00075985	DRR103642	Belgium	Human	EH2251_Belgium_human
PRJNA319494	SAMN05504941*	SRR6154941	Canada	Bovine	OLC0637_Canada_bovine
PRJNA309770	SAMN04420181*	SRR6061322	Germany	Human	126814_Germany_human
PRJNA301341	SAMN04254589	SRR3110022	Canada	Human	EC120246_Canada_human
PRJEB10700	SAMEA3529294	ERR1010233	Denmark	Human	AA044_Denmark_human
PRJDB5571	SAMD00075758	DRR103415	France	Human	99109_France_human
PRJDB5571	SAMD00075757	DRR103414	France	Human	02113_France_human
PRJDB5571	SAMD00075759	DRR103416	France	Human	03139_France_human
PRJNA230969	SAMN05605330	SRR5330864	Germany	Human	MOD1EC2814_Germany_human
PRJNA230969	SAMN05607379	SRR5330926	Germany	Human	MOD1EC1664_Germany_human
PRJDB5571	SAMD00075765	DRR103422	Italy	Bovine	ED80_Italy_bovine
PRJNA230969	SAMN06555271	SRR5336246	Mexico	Food	MOD1EC5336_Mexico_food
PRJEB23743	SAMEA104413463	ERR2210764	Poland	Human	10016_Poland_human
PRJDB5571	SAMD00075756	DRR103413	Switzerland	Human	TC6167_Switzerland_human
PRJDB5571	SAMD00075762	DRR103419	UK	Human	H19_UK_human
PRJNA419720	SAMN08095914	SRR6321366	UK	Human	2M8BS8_UK_human
PRJNA419720	SAMN08095913	SRR6321365	UK	Human	I20VK7_UK_human
PRJNA419720	SAMN08095930	SRR6321271	UK	Human	KLAV92_UK_human
PRJNA419720	SAMN08095938	SRR6321331	UK	Human	HKCVSH_UK_human
PRJEB4681	SAMEA2204500	ERR435109	UK	Human	ECO0283_UK_humanebris
PRJNA315192	SAMN06030740	SRR5031110	UK	Bovine	211644_UK_bovine
PRJDB5571	SAMD00075752	DRR103409	USA	Human	TC6165_USA_human
PRJDB5571	SAMD00075754	DRR103411	USA	Bovine	TC6169_USA_bovine
PRJDB5571	SAMD00075747	DRR103404	USA	Bovine	TC3486_USA_bovine
PRJDB5571	SAMD00075748	DRR103405	USA	Bovine	TC3630_USA_bovine
PRJDB5571	SAMD00075749	DRR103406	USA	Bovine	TC3656_USA_bovine
PRJDB5571	SAMD00075751	DRR103408	USA	Bovine	TC4219_USA_bovine
PRJDB5571	SAMD00075753	DRR103410	USA	Human	TC6168_USA_human
PRJNA218110	SAMN04498710	SRR3178054	USA	Human	2009C3689_USA_human
PRJNA218110	SAMN04498712	SRR3178056	USA	Human	2009C3996_USA_human
PRJNA218110	SAMN04633589	SRR3371771	USA	Human	2009C4747_USA_human
PRJNA218110	SAMN04633622	SRR3371781	USA	Human	2010C3051_USA_human
PRJNA218110	SAMN04625574	SRR3360206	USA	Human	2010C3902_USA_human
PRJNA218110	SAMN04625585	SRR3360195	USA	Human	2010C4430_USA_human
PRJNA218110	SAMN04625563	SRR3360216	USA	Human	2011C3270_USA_human
PRJNA218110	SAMN04913811	SRR4113678	USA	Human	PNUSAE002149_USA_human
PRJNA218110	SAMN04913824	SRR4300141	USA	Human	PNUSAE002166_USA_human
PRJNA218110	SAMN04625466	SRR3360241	USA	Human	2011C3506_USA_human
PRJNA218110	SAMN04578418	SRR3290033	USA	Human	2012C3101_USA_human
PRJNA218110	SAMN04495854	SRR3171841	USA	Human	2012C3912_USA_human
PRJNA218110	SAMN04578419	SRR3290038	USA	Human	2012C3102_USA_human
PRJNA218110	SAMN04498549	SRR3178026	USA	Human	2012C4606_USA_human
PRJNA218110	SAMN04192188	SRR3040537	USA	Human	PNUSAE001578_USA_human

BioProject	BioSample	SRA run	Country	Source	Current_study_ID
PRJNA218110	SAMN03838116	SRR2481234	USA	Human	PNUSAE000885_USA_human
PRJNA218110	SAMN04227723	SRR3040532	USA	Human	PNUSAE001573_USA_human
PRJNA218110	SAMN04075848	SRR2417066	USA	Human	PNUSAE001154_USA_human
PRJNA218110	SAMN03272820	SRR1738019	USA	Human	PNUSAE000133_USA_human
PRJNA218110	SAMN03151532	SRR1635531	USA	Human	PNUSAE000002_USA_human
PRJNA218110	SAMN03840334	SRR2121025	USA	Human	PNUSAE000539_USA_human
PRJNA218110	SAMN04075513	SRR2415794	USA	Human	PNUSAE001379_USA_human
PRJNA218110	SAMN04075843	SRR2415808	USA	Human	PNUSAE001373_USA_human
PRJNA218110	SAMN03775204	SRR2481344	USA	Human	PNUSAE000779_USA_human
PRJNA218110	SAMN04500985	SRR3189440	USA	Human	PNUSAE002228_USA_human
PRJNA218110	SAMN04588711	SRR3371981	USA	Human	PNUSAE002615_USA_human
PRJNA218110	SAMN05209084	SRR3644551	USA	Human	PNUSAE003275_USA_human
PRJNA218110	SAMN05203326	SRR3644569	USA	Human	PNUSAE003211_USA_human
PRJNA218110	SAMN07373072	SRR5870554	USA	Human	PNUSAE008468_USA_human
PRJNA230969	SAMN05605272	SRR5330849	USA	Human	MOD1EC1750_USA_human
PRJNA230969	SAMN04902887	SRR3465501	USA	Other animal	MOD1EC6201_USA_other animal
PRJNA230969	SAMN05605257	SRR5330857	USA	Human	MOD1EC1919_USA_human
PRJNA230969	SAMN05591573	SRR5330824	USA	Human	MOD1EC550_USA_human
PRJNA268206	SAMN06256289	SRR5202193	USA	Bovine	FSIS1609416_USA_bovine
PRJNA268206	SAMN04908471	SRR3457631	USA	Bovine	FSIS1606391_USA_bovine
PRJNA268206	SAMN06127049	SRR5091629	USA	Bovine	FSIS1608854_USA_bovine
PRJNA268206	SAMN06127045	SRR5091628	USA	Bovine	FSIS1608722_USA_bovine
PRJNA268206	SAMN07237071	SRR5683240	USA	Bovine	FSIS1701668_USA_bovine
PRJNA268206	SAMN06700862	SRR5441623	USA	Bovine	FSIS1710186_USA_bovine
PRJNA268206	SAMN07987839	SRR6265848	USA	Bovine	FSIS11704781_USA_bovine
PRJNA268206	SAMN07774189	SRR6158105	USA	Bovine	FSIS21720313_USA_bovine
PRJNA218110	SAMN05294505	SRR3883019	USA	Human	PNUSAE003398_USA_human
PRJNA218110	SAMN02352904	SRR3213940	USA	Human	643464_USA_human
PRJNA218110	SAMN02352964	SRR3371784	USA	Human	2010C3472_USA_human
PRJNA218110	SAMN08129177	SRR6359280	USA	Human	PNUSAE011184_USA_human
PRJNA230969	SAMN05439479	SRR3988028	USA	Food	MOD1EC6029_USA_food
PRJNA230969	SAMN05605269	SRR5330852	USA	Human	MOD1EC1753_USA_human
PRJNA230969	SAMN05605332	SRR5185399	Germany	Human	MOD1EC2812_Germany_human
PRJNA230969	SAMN05605331	SRR5185402	Germany	Human	MOD1EC2813_Germany_human
PRJNA230969	SAMN05605263	SRR5185394	Germany	Bovine	MOD1EC1763_Germany_bovine
PRJNA230969	SAMN03743659	SRR2176280	Canada	Human	CFSAN033951_Canada_human
PRJDB5571	SAMD00075828	DRR103485	Japan	Human	M01_Japan_human
PRJDB5571	SAMD00075829	DRR103486	Japan	Human	M02_Japan_human
PRJDB5571	SAMD00075830	DRR103487	Japan	Human	M03_Japan_human
PRJDB5571	SAMD00075832	DRR103489	Japan	Human	M05_Japan_human
PRJDB5571	SAMD00075905	DRR103562	Japan	Human	O01_Japan_human
PRJDB5571	SAMD00075998	DRR103655	Japan	Human	T02_Japan_human
PRJDB5571	SAMD00075999	DRR103656	Japan	Human	T03_Japan_human
PRJDB5571	SAMD00075911	DRR103568	Japan	Human	O07_Japan_human
PRJDB5571	SAMD00075785	DRR103442	Japan	Human	F02_Japan_human
PRJDB5571	SAMD00076003	DRR103660	Japan	Human	T08_Japan_human
PRJDB5571	SAMD00075908	DRR103565	Japan	Human	O04_Japan_human
PRJDB5571	SAMD00075912	DRR103569	Japan	Human	O08_Japan_human
PRJDB5571	SAMD00075786	DRR103443	Japan	Human	F03_Japan_human
PRJDB5571	SAMD00076004	DRR103661	Japan	Human	T09_Japan_human
PRJDB5571	SAMD00076010	DRR103667	Japan	Human	T16_Japan_human
PRJDB5571	SAMD00075789	DRR103446	Japan	Human	F06_Japan_human
PRJDB5571	SAMD00075848	DRR103505	Japan	Human	M21_Japan_human
PRJDB5571	SAMD00075918	DRR103575	Japan	Human	O16_Japan_human
PRJDB5571	SAMD00076017	DRR103674	Japan	Human	T23_Japan_human
PRJDB5571	SAMD00075930	DRR103587	Japan	Human	O29_Japan_human
PRJDB5571	SAMD00075927	DRR103584	Japan	Human	O25_Japan_human
PRJDB5571	SAMD00075852	DRR103509	Japan	Human	M25_Japan_human
PRJDB5571	SAMD00075793	DRR103450	Japan	Human	F10_Japan_human
PRJDB5571	SAMD00075728	DRR103385	Japan	Bovine	Aki01_Japan_bovine
PRJDB5571	SAMD00075856	DRR103513	Japan	Human	M30_Japan_human
PRJDB5571	SAMD00076025	DRR103682	Japan	Human	T32_Japan_human
PRJDB5571	SAMD00075936	DRR103593	Japan	Human	O35_Japan_human
PRJDB5571	SAMD00076024	DRR103681	Japan	Human	T31_Japan_human
PRJDB5571	SAMD00076026	DRR103683	Japan	Human	T33_Japan_human
PRJDB5571	SAMD00076028	DRR103685	Japan	Human	T35_Japan_human
PRJDB5571	SAMD00075799	DRR103456	Japan	Human	F17_Japan_human
PRJDB5571	SAMD00076030	DRR103687	Japan	Human	T39_Japan_human
PRJDB5571	SAMD00076032	DRR103689	Japan	Human	T41_Japan_human

BioProject	BioSample	SRA run	Country	Source	Current_study_ID
PRJDB5571	SAMD00075857	DRR103514	Japan	Human	M31_Japan_human
PRJDB5571	SAMD00075947	DRR103604	Japan	Human	O46_Japan_human
PRJDB5571	SAMD00075859	DRR103516	Japan	Human	M33_Japan_human
PRJDB5571	SAMD00076034	DRR103691	Japan	Human	T43_Japan_human
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BioProject	BioSample	SRA run	Country	Source	Current_study_ID
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PRJNA259827	SAMN03703965	SRR2035374	UK	Human	63713_UK_human
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PRJNA268206	SAMN03922101	SRR2126001	USA	Bovine	FSIS1500781_USA_bovine
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PRJNA284656	SAMN03704963	36084†	France	Human	36084_France_human
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PRJNA230969	SAMN05425824	SRR3938671	USA	Food	FDA00010430_USA_food
PRJNA230969	SAMN05452918	SRR3987970	USA	Avian	MOD1EC5703_USA_avian
PRJNA230969	SAMN05605333	SRR4340534	USA	Human	MOD1EC2790_USA_human

*Origin of isolate found by contacting submitter and original source.

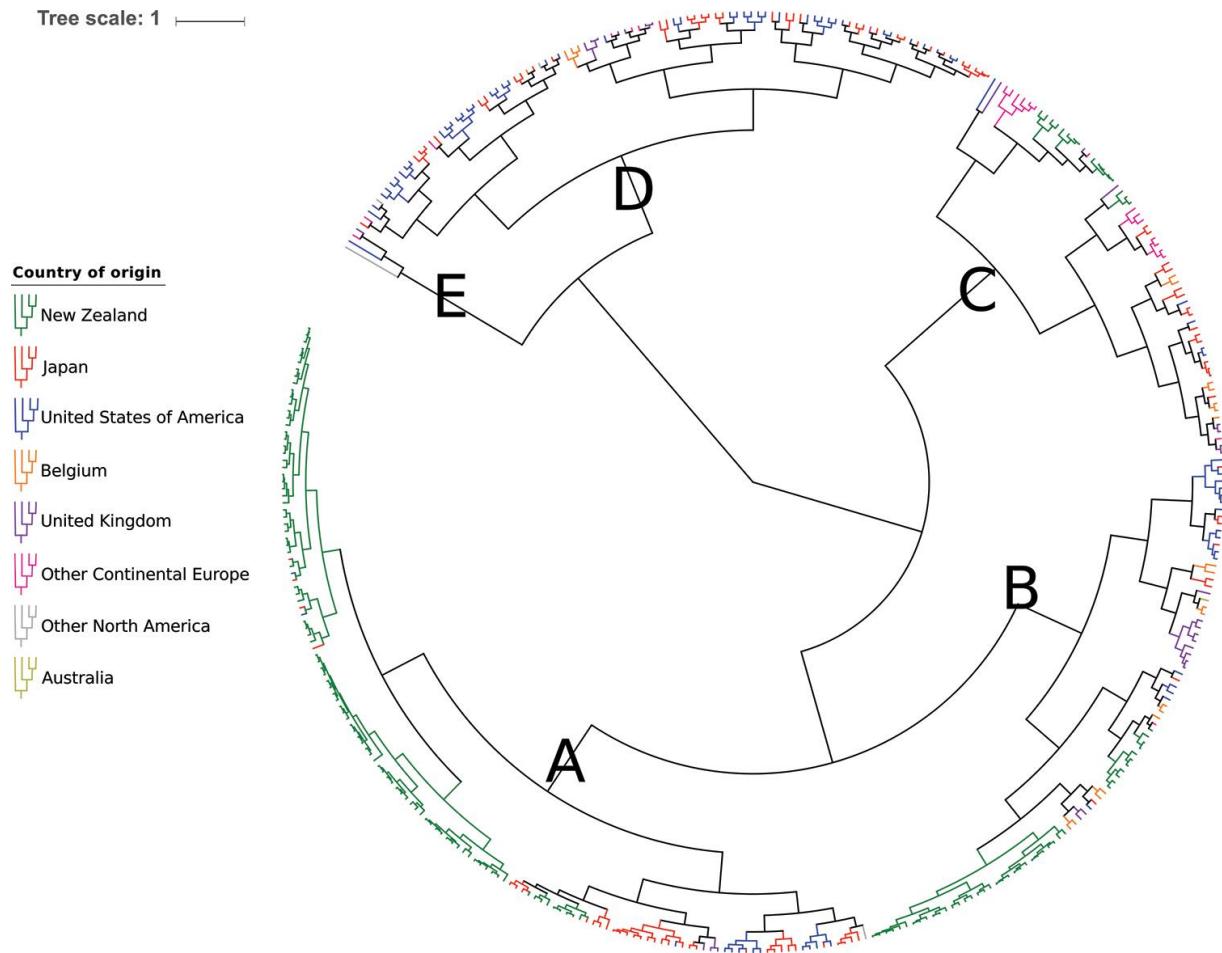
†Raw sequence data provided by submi.

Appendix 1 Table 3. Virulence genes (n = 192) identified in serogroup O26 isolates (n = 404), annotated with function of gene

Gene name	Function
acpXL	Acyl carrier protein
afaA	Major fimbrial subunit of aggregative adherence fimbria II
afaB-I	Chaperone protein
afaC-VII	Putative AfaC-VIII usher protein
afaD-VII	Invasin protein
afaE-VII	Adhesin
afaG-VII	Adhesin
agg3B	Putative invasin protein
aslA	Putative arylsulfatase
astA	Heat-stable enterotoxin 1
cesAB	Chaperone for EspA and EspB
cesD	Chaperone for EspD and EspB
cesD2	Chaperone for EspD
cesF	Chaperone CesF
cesL	Chaperone for SepL
cesT	Chaperone for Tir
cheY	Chemotaxis proteinotaxis protein
chuS	Putative heme/hemoglobin transport protein/me/hemoglobin transport protein
chuU	iron ABC transporter permease
chuV	ATP binding hydrophilic protein
chuW	Coproporphyrinogen III oxidase
chuY	Hypothetical protein
cif	Type III secreted effector
cnf1	cytotoxic necrotizing factor 1
csgA	Curlin major subunit
csgB	Minor curlin subunit precursor
csgC	Curli assembly proteinoagglutination protein
csgD	Operon transcriptional regulatory protein
csgE	Curli production assembly/transport protein
csgF	Curli production assembly/transport protein
csgG	Curli production assembly/transport protein
eae	Intimin
east1	EAST1
efa1	EHEC factor for adherence
entA	2,3-dihydroxybenzoate-2,3-dehydrogenase
entB	Isochorismatase
entC	Isochorismate synthase 1
entD	Phosphopantetheinyl transferase component of enterobactin synthase multienzyme complex
entE	2,3-dihydroxybenzoate-AMP ligase component of enterobactin synthase multienzyme complex
entF	Enterobactin synthase multienzyme complex component, ATP-dependent
entS	Enterobactin exporter
escC	Type III secretion system secretin
escD	Type III secretion system outer MS ring protein
escE	Chaperone for EscF
escF	Type III secretion system needle filament protein
escG	Chaperone for EscF
escI	Type III secretion system inner rod component
escJ	Type III secretion system inner MS ring protein
escL	Negative regulator
escN	ATPase
escO	Positive regulator
escP	Type III secretion system needle length regulator
escR	Type III secretion system minor export apparatus protein
escS	Type III secretion system minor export apparatus protein
escT	Type III secretion system minor export apparatus protein
escU	Type III secretion system export apparatus switch protein
escV	Type III secretion system major export apparatus protein
espA	Type III secretion system
espD	Type III secretion system
espF	Type III secretion system
espG	Type III secretion system
espH	Type III secretion system
espJ	Prophage-encoded type III secretion system effector
espK	ESX-1 type VII secretion system
espL1	serine protease autotransporters of Enterobacteriaceae (SPATE)
espL2	serine protease autotransporters of Enterobacteriaceae (SPATE)

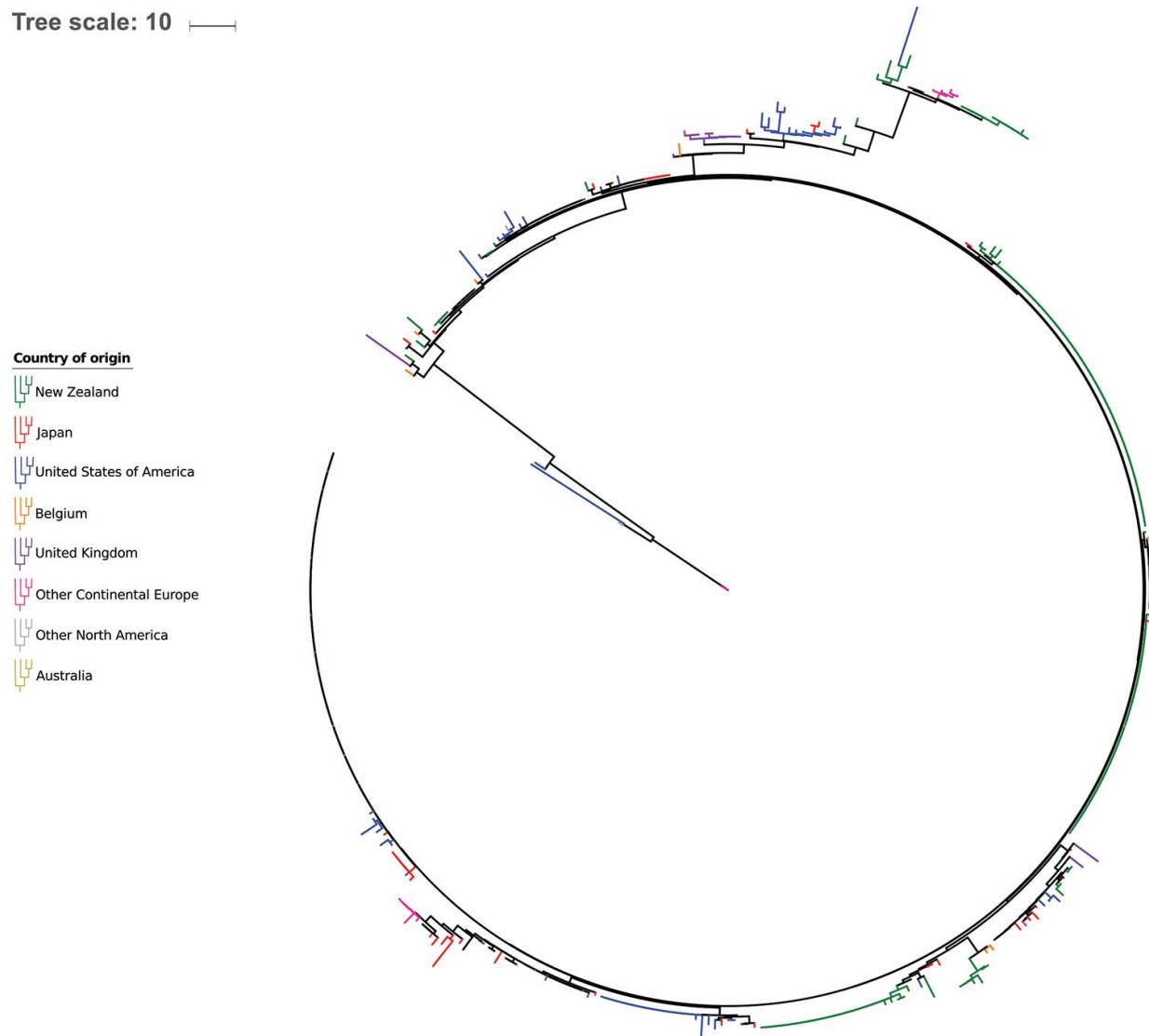
Gene name	Function
espL4	serine protease autotransporters of Enterobacteriaceae (SPATE)
espM1	BfpT-regulated chaperone
espM2	Putative T3SS effector protein
espN	T3SS effector
espP	Extracellular serine protease plasmid-encoded
espR1	Putative type III secreted effector
espW	T3SS effector
espX1	Putative type III secreted effector
espX2	Putative type III secreted effector
espX4	Putative type III secreted effector
espX5	Putative type III secreted effector
espX6	Putative type III secreted effector
espX7/nleL	Putative type III secreted effector
espY1	Apoptosis/cell cycle regulation
espY2	T3SS effector-like protein
espY3	Putative type III secreted effector
espY4	Putative type III secreted effector
etgA	T3SS-associated peptidoglycan lytic enzyme
etpB	Two-partner secretion transporter
faeC	K88 fimbrial protein
faeD	Outer membrane usher protein
faeE	Acyl-coenzyme A dehydrogenase
faeF	K88 minor fimbrial protein
faeH	K88 minor fimbrial protein
fael	K88 minor fimbrial protein
faeJ	K88 minor fimbrial protein
fdeC	Adhesin
fepA	Ferrienterobactin outer membrane transporter
fepB	Ferrienterobactin ABC transporter periplasmic binding protein
fepC	Ferrienterobactin ABC transporter ATPase
fepD	Ferrienterobactin ABC transporter permease
fepG	Iron-enterobactin ABC transporter permease
fes	Enterobactin/ferric enterobactin esterase
fimA	Type-1 fimbrial protein
fimB	Type 1 fimbriae Regulatory protein
fimC	Chaperone protein
fimD	Outer membrane usher protein
fimE	Type 1 fimbriae Regulatory protein
fimF	Type 1 fimbrial minor component
fimG	Type 1 fimbrial minor component
fimH	Type 1 fimbrial adhesin precursor
fimI	Type 1 pilus biosynthesis fimbrial protein
flgD	Flagellar basal body rod modification protein
flgG	Flagellar basal-body rod protein
flgH	Flagellar L-ring protein precursor
flhA	Flagellar biosynthesis protein
fliG	Flagellar motor protein
fliI	Flagellum-specific ATP synthase
fliM	Flagellar motor switch protein
fliN	Flagellar motor switch protein
fliP	Flagellar biosynthesis protein
fyuA	Yersiniabactin receptor FyuA
gspC	General secretion pathway protein C
gspD	General secretion pathway protein D
gspE	General secretion pathway protein E
gspF	General secretion pathway protein F
gspG	General secretion pathway protein G
gspH	General secretion pathway protein H
gspI	General secretion pathway protein I
gspJ	General secretion pathway protein J
gspK	General secretion pathway protein K
gspL	General secretion pathway protein L
gspM	General secretion pathway protein M
gtrA	Bactoprenol-linked glucose translocase/flippase
gtrB	Bactoprenol glucosyl transferase
hlyA	Hemolysin transport protein
hlyB	Hemolysin transport protein
hlyC	Hemolysin transport protein
hlyD	Hemolysin transport protein

Gene name	Function
iroB	Glucosyltransferase
iroC	ABC transporter
iroD	Siderophore esterase
iroE	Siderophore esterase
iron	Enterobactin siderophore receptor protein
irp1	Yersiniabactin polyketide synthase HMWP1
irp2	Yersiniabactin non-ribosomal peptide synthetase HMWP2
iucA	Aerobactin siderophore biosynthesis protein
iucB	Aerobactin siderophore biosynthesis protein
iucC	Aerobactin siderophore biosynthesis protein
iucD	L-lysine 6-monooxygenase
iutA	Ferric aerobactin receptor precursor
map	Rho guanine exchange factor
nleA	Non-LEE-encoded effector
nleB1	Non-LEE encoded effector B
nleB2	Non-LEE encoded effector B
nleC	Non-LEE encoded effector C
nleD	Type III secretion system effector
nleE	T3SS secreted effector protein
nleF	Effector protein
nleG7	Non-LEE-encoded type III effector
nleH1	Non-LEE-encoded type III effector
nleH2	Non-LEE-encoded type III effector
ompA	Outer membrane protein
ospG	Type III secretion system effector kinase
paa	Outer membrane adhesin
sepD	Type III secretion system secretion switch protein
sepL	Type III secretion system secretion gatekeeper
sepQ/escQ	Type III secretion system C ring protein
sepZ/espZ	Type III secretion system effector
set1A	Toxin subunit
set1B	Toxin subunit
shuA	Outer membrane heme/hemoglobin receptor
shuT	Periplasmic binding protein
shuX	Shu locus protein
spaQ	Type III secretion system minor export apparatus protein
stcE	Metalloprotease
stx1B	Shiga toxin 1, subunit B
stx2A	Shiga toxin 2, subunit A
stx2B	Shiga toxin 2, subunit B
stx1A	Shiga toxin 1, subunit A
toxB	Toxin B
vat	Vacuolating autotransporter toxin
yagV/ecpE	E. coli common pilus chaperone
yagW/ecpD	Polymerized tip adhesin of ECP fibers
yagX/ecpC	E. coli common pilus usher
yagY/ecpB	E. coli common pilus chaperone
yagZ/ecpA	E. coli common pilus structural subunit
ybtA	Yersiniabactin transcriptional regulator
ybtE	Yersiniabactin biosynthesis salicyl-AMP ligase
ybtP	Yersiniabactin ABC transporter ATP binding/permease protein
ybtQ	Yersiniabactin ABC transporter ATP binding/permease protein
ybtS	Yersiniabactin biosynthesis salicylate synthase
ybtT	Yersiniabactin biosynthesis thioesterase
ybtU	Yersiniabactin biosynthesis oxidoreductase
ybtX	Yersiniabactin-associated zinc MFS transporter
ykgK/ecpR	Regulator protein

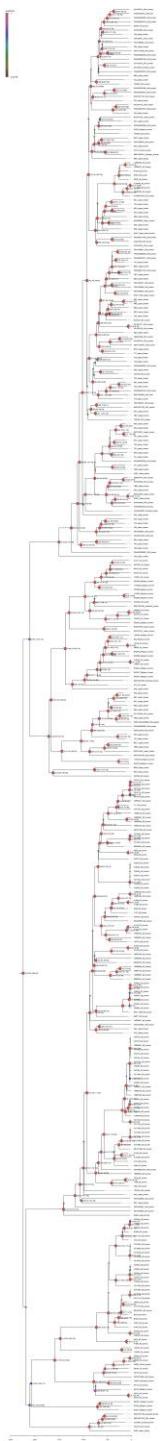


Appendix 1 Figure 1. RaxML maximum-likelihood hierarchical set tree of pangenome elements of *Escherichia coli* serogroup O26 isolates ($n = 404$), with real branch lengths and annotated by country.

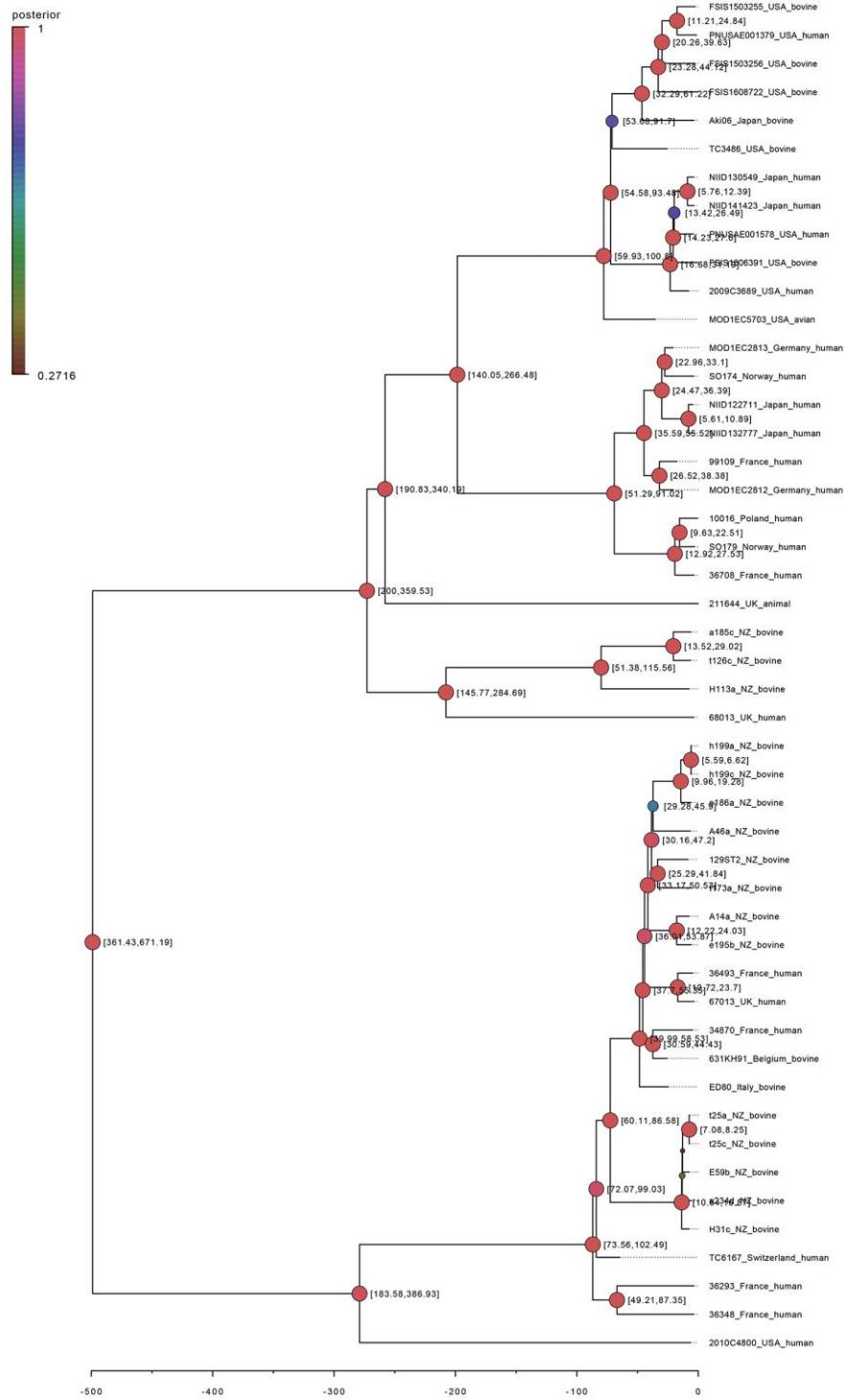
Tree scale: 10



Appendix 1 Figure 2. Neighbor-joining tree of virulence genes ($n = 192$) of *Escherichia coli* serogroup O26 isolates ($n = 404$) with real branch lengths annotated by country.



Appendix 1 Figure 3. Maximum clade credibility tree of time of most recent common ancestor (TMRCA) analysis of *Escherichia coli* serogroup O26 sequence type 21 (ST-21) isolates ($n = 344$), annotated with posterior probability of tree branches. All convergence dates are annotated with 95% HPD intervals from the age of the newest isolate (2017.5 in decimal years).



Appendix 1 Figure 4. Maximum clade credibility tree of time of most recent common ancestor (TMRCA) analysis of *Escherichia coli* serogroup O26 sequence type 29 (ST-29) isolates ($n = 48$), annotated with posterior probability of tree branches. All convergence dates are annotated with 95% HPD intervals from the age of the newest isolate (2017.0411 in decimal years).